believes that it would have been obvious to modify the teachings of Hoshimaru, *et al.* and Prasad, *et al.* with the substitution of human mesencephalic cells as taught by Boss, *et al.* The Examiner further believes it would be obvious to characterize immortalized human mesencephalic cells as described by Gallyas, *et al.* because GABA and dopamine are neurotransmitters of interest.

Applicants respectfully disagree with the Examiner's assertions and conclusions, and traverse this ground of rejection. The initial burden is on the Examiner to make out a *prima facie* case of obviousness. MANUAL OF PATENT EXAMINING PROCEDURE (MPEP) § 2142, at 2100-96. To establish a *prima facie* case of obviousness, three criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge available to one of skill in the art to combine the references. Second, there must be a reasonable expectation of success. Finally, the prior art references, when combined, must teach or suggest all of the claim limitations. MPEP § 2142, at 2100-97. Applicants respectfully assert that the combination of references cited by the Examiner fails at least two of these criteria. First, the combination fails to teach every element of the claimed invention. Second, the Examiner has proffered no evidence that the cited combination provides a reasonable expectation of success in practicing the present invention. Thus, the cited references cannot in combination render the invention obvious, and consequently the Examiner has not made out the required *prima facie* case of obviousness.

Applicants restate the fact that prior to their development of conditionally-immortalized human mesencephalon neural precursor cells, no such cells had been developed in the art, despite many years of research in this area. The Examiner has not addressed the failure of others in the art to generate these human cells using methods and materials known in the art at the time the invention was made. Had the development of such human cell lines been obvious, they most certainly would have been produced before Applicants developed the present invention. The failure of others in the relevant art to develop the invention is important evidence that the invention is non-obvious.

The Examiner has not established that the combination of references provides a reasonable expectation of success in practicing the present invention. The Examiner asserts that "[i]t would have been obvious to one of ordinary skill in the art . . . to substitute the immortalized rat neuronal progenitor cells as taught by Hoshimaru et al and Prasad et al with human mesencephalon neuron progenitors [*sic*] cells as taught by Boss et al." March 16, 1999 Office Action, at 4. On the contrary, a person of skill in the art would not have expected the method of

Hoshimaru, *et al.*, using rat hippocampal cells, to permit the conditional immortalization of human mesencephalic neuronal progenitor cells. Cells from different regions of the CNS are known to respond differently to attempts to generate immortalized cell lines, and the Examiner has pointed to nothing in the cited references that would demonstrate that a person of skill in the art making the substitution would have a reasonable expectation of success. The assertion of obviousness made by the Examiner is, therefore, essentially that it would be obvious to *try* substituting the rat cells used in Hoshimaru, *et al.* with human cells. However, "obvious to try" is an improper basis for a §103(a) rejection. *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988). Thus, the invention as embodied in the present invention cannot be rendered obvious by Hoshimaru, *et al.*

It would not be obvious that the methods of Hoshimaru, *et al.* and Prasad, *et al.* would work with human mesencephalon neural progenitor cells because the methods taught by these two references differ substantially from that of the present invention. For example, different growth factors are used within the growth media for proliferation and differentiation. For proliferation, Hoshimaru, *et al.* use only FGF-2. *See* p. 1519, col. 1, ¶ 3. The methods of the present invention, however, use medium containing EGF or PDGF. Prasad, *et al.* do not use growth factors. *See* p. 597, col. 2, ¶ 2. Furthermore, Hoshimaru, *et al.* use DMEM, a minimal medium, and Ham's F-12, a defined medium, with N2 supplement. *See* p. 1519, col. 1, ¶ 3. Prasad, *et al.* use only the defined medium MCDB-153, which contains different ingredients than DMEM and F-12, followed after one year by F12 medium. *See* p. 597, col. 2, ¶ 2. Prior to the present invention, those of ordinary skill in the art would have had no basis for determining which elements of the Hoshimaru, *et al.* or Prasad, *et al.* teachings to retain, and which to alter, for use with human mesencephalic cells, and the cited references do not provide such a basis. It is not obvious, therefore, given the vastly different components used by each, that the use of the growth media described by Hoshimaru, *et al.* or Prasad, *et al.* would result in the generation of conditionally-immortalized human mesencephalon neural precursor cells as recited in the present claims.

Furthermore, the culture of the cell lines as taught by Prasad, *et al. requires* the use of tissue culture dishes precoated with a specialized substrate, consisting of bovine serum albumen, fibronectin and collagen. *See* p. 597, col. 2, ¶2. There is no suggestion in Prasad, *et al.* that this surface would be necessary for the proliferation of mesencephalon progenitor cells. This is because Prasad, *et al.*, do not teach or disclose rat neuronal progenitor cells, but an

immortalized neuronal cell line. Clearly, the cells of Prasad, *et al.* differ substantially from those of the current invention. Consequently, there can be no expectation that, as the Examiner suggests, it would be obvious to substitute the *cell line* cells of Prasad, *et al.* with the *progenitor* cells of the present invention.

Boss, *et al.* and Gallyas, *et al.* do not remedy these deficiencies. Boss, *et al.* is directed to the isolation and proliferation of nonimmortalized human neuron progenitor cells, while Gallyas is directed to the characterization of immortalized mouse neural cell lines by measuring the concentration of certain neurotransmitters. Neither reference discloses the Applicants' conditions suitable for producing conditionally immortalized human mesencephalic neural progenitor cells; thus, their citation in combination with Hoshimaru, *et al.* and Prasad, *et al.* cannot render the present invention obvious.

Contrary to the Examiner's assertions, Boss, *et al.* does *not* describe the production of monolayers of cells as recited by the current invention[1]. Boss, *et al.* specifically states that "[g]ross examination of typical neuron progenitor cell 'monolayer' cultures reveals interconnected three-dimensional structures, rather than the usual two-dimensional monolayer observed with most cell lines." Col. 6, ll. 4-7. It is quite clear that Boss, *et al.* do *not* consider these "monolayers" of progenitor cells to be monolayers, as persons of skill in the art recognize the term, but clumps of cells. Indeed, the method disclosed by Boss, *et al.* for producing "monolayers" states specifically that one must titurate the cells with a BSA-coated Pasteur pipet as the "cells clump rapidly upon standing." Col. 11, ll. 54-56. This is not characteristic of cells that would form a true monolayer as recognized by persons of skill in the art. Applicants' progenitor cells, in sharp contrast, produce true monolayers. This difference was pointed out to the Examiner previously. *See* Amendment mailed June 30, 2000, at 4. The Examiner appears not to have considered this important distinction, instead merely restating the belief that Boss, *et al.* teaches monolayer culture. *See* October 3, 2000 Office Action at 3.[2] Again, in making out a *prima facie* case of obviousness, it is up to the Examiner to *factually* support such a conclusion.

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[1] Applicants wish to point out that they have argued that the "monolayers" disclosed in Boss, *et al.* grow *three*-dimensionally, not *two*-dimensionally, as the Examiner suggests; *see* Office Action mailed October 3, 2000, at 2. Applicants, rather, have argued that the progenitor cells of the present invention grow in two-dimensional monolayers.

[2] The Examiner's citation to sections of Boss, *et al.* that ostensibly teach "monolayer" culture is somewhat confusing, as several of these sections do not appear to relate to monolayer cultures.

MPEP § 2142, at 2100-96. Boss, *et al.* go on to state that "[o]ver time, cells begin to migrate from these [three-dimensional] structures and form typical two-dimensional monolayers in which differentiating neurons and glia can be observed." Col. 6, ll. 9-12. Again, it is clear here that the "monolayer" is formed *not* by the progenitor cells of the present invention, but by differentiating cells migrating from the progenitor cell clumps. Boss, *et al.* therefore fails to teach progenitor cells that grow in true monolayers, and therefore cannot be combined with either Hoshimaru, *et al.* or Prasad, *et al.* to render the present invention obvious.

The Examiner cites Gallyas, *et al.* as teaching the characterization of mouse immortalized neuronal cell lines by measuring the concentration of various neurotransmitters such as "GABAergic" [*sic*] and dopamine. Applicants respectfully point out that the citation of Gallyas, *et al.* for this teaching is irrelevant because none of the Applicants claims are directed to the characterization of immortalized cell lines by measuring the concentration of neurotransmitters. Rather, the invention is directed to human mesencephalon neural progenitor cell lines that are *capable of* differentiation into GABAergic and dopaminergic cells. Gallyas, *et al.*, in combination with Hoshimaru, *et al.* and Prasad, *et al.*, do not teach the immortalization of a human mesencephalon neuronal precursor cell, wherein the cell is capable of differentiating into a dopaminergic or GABAergic neurons. Thus, this combination of references does not support the contention that the present invention is obvious.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks. No fee is believed due. If a fee is required in connection with this Response, please charge Pennie & Edmonds LLP Deposit Account Number 16-1150 for the appropriate amount.

Respectfully submitted,

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Date March 26, 2001

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EXHIBIT A

MARKED VERSION OF THE CLAIMS

U.S PATENT APPLICATION SERIAL NO. 09/134,771

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1. (Twice amended) A method for producing a conditionally-immortalized human mesencephalon neural precursor cell, comprising:

(a) transfecting human mesencephalon cells plated on a first surface and in first growth medium that permits proliferation with DNA encoding a selectable marker and an externally regulatable growth-promoting protein; and

(b) selecting an adherent monolayer of the transfected cells on a second surface and in a second serum-free growth medium that permits attachment and proliferation, wherein the second serum-free growth medium comprises EGF or PDGF, and therefrom producing a conditionally-immortalized human mesencephalon cells in which the growth-promoting protein is regulated by an external factor, such that suppression of the growth promoting protein results in differentiation of the cell into neurons.